B

Addendum to Page 000019

Analytical Methods

The test methods used for the analysis of Neobee® (Medium Chain Triglycerides) are listed below, along with the corresponding method numbers.

* Replacement Method Enclosed

Stepan Company Analytical Method

Stepan Company
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SM 006-A
Total Pages: 4

APHA, Gardner, % Transmission, and Lovibond Color by LCS or LICO 300 Colorimeter

Specification Method	Accepted: 10/22/2003	Supercedes:09/04/2002
Author:B. Eskalis	Aprv'd: (Anl) Dave Mc	

SCOPE: APHA, Gardner, % Transmission, and Lovibond color determine the color of edible oils and animal/vegetable fat. A Liquid Color Spectrophotometer (LCS) instrument can make transmission measurements of liquid samples by selecting the measuring scale and type of cuvette. The LCS measurements meet ASTM and DIN specifications including Hazen Gardner and iodine values (see Remark 1).

A LICO or Lange spectrophotometer is an acceptable instrument for this method.

SUMMARY: The sample is placed in a cuvette and positioned in the instrument. The color is read directly from the instrument (see Remarks 2 through 6).

SAFETY: This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:
1. Liquid Color Spectrophotometer, LCS
2. LICO 300 Colorimeter
3. Cuvette, 11 mm round glass, 10 mm square glass or plastic, or 50 mm square glass (see Remark 7)

PROCEDURE:

A. SAMPLE PREPARATION:

1. Eliminate sample turbidity by heating or filtering.

2. Sample must be liquid and free of particles, solids or bubbles.

B. SAMPLE COLOR DETERMINATION by LCS COLORIMETER:

1. Press key "C". The selected color system will be indicated on the display.

2. Pour the sample into the cuvette and place into the measuring compartment (see Remarks 2 and 3).

3. Press key "MEAS". After measuring the color, the values of the sample will appear on the display.

4. Press key "DIM" until the desired color scale is shown on the display.

5. "G" for Gardner color measurement.
"H" for Hazen or APHA color measurement.
"Ly & Lr" for Lovibond color measurement with 5 1/4" scale (see Remark 8).
"Ly & Lr" for Lovibond color measurement with 1" scale (see Remark 8).

C. SAMPLE COLOR DETERMINATION by LICO 300 COLORIMETER:

Calibrate the instrument as described in SOP LICO 300 "Calibrating the Instrument" using deionized water as a reference standard.

1. Fill a cuvette, similar to the calibration cuvette, with sample. Sonicate the sample, if necessary.

2. Wipe the sides of the cuvette with a Kimwipe. Place the cuvette into the measurement compartment and close the flap. The instrument will automatically determine the sample color.

3. The sample color values will appear on the display. Press the "DIM" key until the desired color value is shown on the display.

4. For additional samples, remove the previous sample cuvette from the measuring cell compartment and fill a clean cuvette, similar to the calibration cuvette, with the sample. Sonicate the sample, if necessary.

5. Wipe the sides of the cuvette with a KIMWIPE. Place the cuvette into the measuring compartment and close the flap. Press the "MEAS" button.

6. The sample color values will appear on the display. Press the "DIM" button until the desired color value ("G" for Gardner) is shown on the display.

7. To measure a sample using a different type of cuvette, refer to section "Calibrating the Instrument" in the SOP LICO 300.

D. % TRANSMISSION DETERMINATION:

1. Refer to the individual specification sheet for sample preparation.

2. Access the photometric mode of absorbance or transmission. Press K4 until K1(CAL) and K2(CONF) is shown on the display.

3. Press K4 until K1(CLOCK), K2(TEXT), and K3(PHOTO) are shown on the display.

4. Press K3 once to display K1(SCAN), K2(ABS), and K3(DIF).

5. % Transmission determination can be made by K2(ABS) or K3(DIF). To determine by absorbance press K2(ABS) then K3(DIF) to enter or change wavelength. Insert the calibration cuvette or reference.

6. Press K1(ZERO), press again to initiate and place the 10 mm sample cuvette and press K1(MEAS). The transmittance will appear on the display.

7. To return to the basic measurement mode, press K1 three times to display K1(CAL), K2(CONF). Press K1(CAL) and proceed as usual for calibration and sample color determination.

PRECISION and ACCURACY:

PRECISION:

APHA COLOR:

These error statements are based on an ASTM round robin study (see Reference 4) conducted with the cooperation of ten member laboratories.

The standard deviation of the method for between laboratory application is 2 platinum-cobalt (APHA color) units.

The standard deviation of the method for within laboratory application is 1 platinum-cobalt (APHA color) unit. Duplicate analyses by a single operator must agree within 2 platinum-cobalt (APHA color) units to be considered acceptable.

GARDNER COLOR:

These error statements are based on an ASTM round robin study (see Reference 5) conducted on 4 samples with the cooperation of 80 laboratories.

The standard deviation of the method for between laboratory application is 0.5 Gardner color number.

The standard deviation of the method for within laboratory application is 0.1 Gardner color number. Duplicate analysis by a single operator must agree within 2/3 of a Gardner color number to be considered acceptable.

LOVIBOND COLOR:

Readings should be within +/- 0.2 red units and +/- 0.4 yellow units (see Reference 6).

% TRANSMISSION:

Reproducibility for the LCS instrument is +/- 0.2% transmission referenced to distilled water (see Reference 7).

For a typical high active sample (> 60% actives) color level of 86%T (see Reference 8):
Average repeatability standard deviation: s(r) = 1.0% (1.1% RSD)
Single Analyst 95% repeatability limit = 0.09%
Method 95% repeatability limit (within laboratory) = 2.9%
Method 95% reproducibility limit (between laboratories) = 3.9%

REMARKS:

1. This spectrophotometric method can replace SM 006-B, APHA COLOR, SM 006-C, GARDNER COLOR, and SM 511-0, LOVIBOND COLOR.
2. Do not use the instrument in direct sunlight.
3. Do not allow water, flammable, or metallic objects to spill into or otherwise be introduced into the instrument.
4. Do not put heavy objects on the housing.
5. Do not use the instrument in areas that are very humid, dusty, or subject to vibration.
6. Cleanliness is of vital importance for accurate measurement results. The cuvette should be clean and air bubbles in the sample should be avoided.
7. Use a 10 mm square cuvette for phosphate esters.
8. Lovibond color results unit measurement is equal to 10 times the red color plus the yellow color.

REFERENCES:

1. The Hazen color value, known as APHA method or Platinum-Cobalt Test Method D 1209.
2. Gardner color ASTM D 1544-80 and DIN ISO 4630.
3. Lovibond color Ly and Lr as used by LCS refer to the Lovibond yellow/red

values determined by instrument model AF900/AF960 which uses 5 1/4" and 1" cuvettes AOCS Co 13 B-45 (1962 Rev.).
4. Annual Book of ASTM Standards, Volume 06.03 (1987), Designation D 1209.
5. Annual Book of ASTM Standards, Volume 06.03 (1987), Designation D 1544-80.
6. AOCS Co-13B-45 (1962 Rev.)
7. LCS Users Guide, Technical Notes, Chapter 4, pp. 4-5.
8. Strnad, J.T., "High Active Paste Methodology: Second Round Robin Results", Stepan Library Document #94102, 7/28/94.

APPENDICES:
Graphics:

Stepan Company Analytical Method

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SM 053-0
Total Pages: 3

Hydroxyl Value Determination by Acetylation

Specification Method	Accepted: 01/21/2004	Supercedes:11/05/2002
Author:A. Murphy	Aprv'd: (Anl) Dave McGyn	

SCOPE:

This method determines the hydroxyl groups in nonionics and polymer glycols, esters, and ethers. It may be applied to the determination of the hydroxyl function in many other substances provided the material contains primary hydroxyl groups.

Primary and secondary amines along with higher fatty acids react with the acetylating reagent to form stable compounds. These interferences will affect the accuracy of the analysis.

An automated NIR method is available for determining the OHV of some Stepan products. Refer to SM 483-0, NIR ANALYSIS of STEPAN PRODUCTS, for complete experimental details.

An automated FTIR method is available for determining the OHV of some Stepan TOXIMUL® block co-polymers and Maywood products. Refer to SM 167-B, FTIR ANALYSIS of STEPAN PRODUCTS, for complete experimental details.

Alternatively, the OHV can be determined potentiometrically using an autotitrator equipped with a glass combination pH electrode.

SUMMARY:

The hydroxyl group is acetylated with a solution of acetic anhydride in pyridine. The excess reagent is decomposed with water and the acetic acid which is formed is titrated with standardized sodium hydroxide solution.

SAFETY:

ACETIC ANHYDRIDE is CORROSIVE, FLAMMABLE, TOXIC, and an IRRITANT. Avoid eye and skin contact. Avoid open flames and sparks. Work in a hood or well ventilated area. Wear proper personal protective equipment.

PYRIDINE is FLAMMABLE, TOXIC, and an IRRITANT. Avoid eye and skin contact. Avoid open flames and sparks. Work in a hood or well ventilated area. Wear proper personal protective equipment.

SODIUM and POTASSIUM HYDROXIDE are CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

DEFINITION(S):

Hydroxyl value is the number of milligrams of KOH equivalent to the hydroxyl content in 1 gram of sample.

APPARATUS:

1. Flask, with ground-glass joints, 250 mL
2. Condensers

3. Hot plate
4. Buret, suitable size

REAGENTS:

1. Pyridine
2. Acetic anhydride, reagent grade
3. Sodium or potassium hydroxide (NaOH or KOH), 2N or 1N, standardized, (1N KOH is used for OHV determination of some Maywood products)
4. Phenolphthalein indicator

PROCEDURE:

A. PREPARATION of ACETYLATING REAGENT:

1. Add exactly 3.5 mL of water to 1 liter of pyridine and mix thoroughly. Add 140 mL of acetic anhydride and mix thoroughly (see Remark 1).

2. Store the acetylating reagent in a brown glass bottle in the dark. The bottle must be labeled with the date of reagent preparation. This reagent has an EXPIRATION DATE and must not be used if more than 2 WEEKS old.

B. HYDROXYL VALUE ANALYSIS:

1. Weigh a sufficient sample to give approximately three-fourths the titration value of the blank into a 250 mL flask having a standard ground-glass joint. See Equation 1, CALCULATIONS section for sample size determination (see Remark 2).

2. Pipet 25 mL of the acetylating reagent to the flask. Grease the ground-glass joints and attach the condenser to the flask. Prepare a blank by pipetting 25 mL of acetylating reagent to another flask not containing any sample (see Remark 3).

3. Reflux the sample and blank solutions for 1 hour on a hot plate (see Remark 4). With the condenser attached, remove the flasks from the heat and rinse the condensers with 30-50 mL of water. Allow the flasks to cool to room temperature.

4. Remove the condensers and rinse the glass joints with a few mL of water. Add a few drops of phenolphthalein indicator and titrate with sodium or potassium hydroxide to the appearance of the pink endpoint which is permanent for at least 15 seconds or the inflection point using a potentiometric titrator.

CALCULATIONS:

$$1.\quad \text{Sample Size, g} = \frac{(7.5)\ (\text{NaOH or KOH N})\ (56.1)}{\text{OHV}}$$

OHV is the expected hydroxyl value. The sample size should not exceed 15g.

$$2.\quad \text{Uncorrected Hydroxyl Value} = \frac{(B - S) \times N \times 56.1}{\text{Sample Weight, g}}$$

Where: B = Titrant mL for the Blank
S = Titrant mL for the Sample
N = Titrant Normality

The true hydroxyl value is calculated from the uncorrected hydroxyl value by adding the acid value or by subtracting the base value.

PRECISION and

ACCURACY: PRECISION:

Ten replicate determinations were performed on 5 samples by 2 operators over a 2 week period in 1 laboratory.

Sample	OHV Average	Std. Dev, %	RSD, %
WECOBEE® FS	3.21	0.25	7.79
WECOBEE® M	3.78	0.19	5.03
NEOBEE® M-20	2.44	0.37	15.16
NEOBEE® M-5	0.90	0.17	18.89
Ethylene Glycol Monostearate (EGMS)	119.69	0.96	0.80

Single determinations were performed on 3 samples by 4 operators in 3 laboratories.

Sample	OHV Average	Std. Dev, %	RSD, %
STEPANPOL® PS-2502-A	260.60	3.57	1.37
STEPANPOL® PS-3152	319.65	2.21	0.69
CEDEPAL® TD-480	157.64	2.96	1.88

REMARKS:

1. It is important that both the amount of water and the order of addition shall be strictly adhered to, otherwise low results are likely to be obtained. The presence of the water inhibits the formation of dark colored resins caused by a reaction between dry pyridine and acetic anhydride during refluxing.
2. When analyzing polyol STEPANPOL® PN-110 weigh 3.5-4.5g of sample. Rinse the condenser of the acetylated sample solution with 50 mL of warm water (50 °C) and titrate while hot on an autotitrator with 1N NaOH. All CASE polyols, STEPANPOL® PN-110, STEPANPOL® PH-56, STEPANPOL® PD-110 LV, STEPANPOL® PD-56, STEPANPOL® PD-200 LV, STEPANPOL® PD-56LV, STEPANPOL® PHN-56 and Agent 2429-39 must be titrated either colorimetrically or potentiometrically while the acetylated solution is hot.
3. This analysis must be done in duplicate along with duplicate blanks for qualifying the method.
4. For samples with hydroxyl values under 150, reflux the sample for 2 hours to complete the acetylation.

REFERENCES:

1. Method originator, G.T.Battaglini
2. Snell and Biffen, Commercial Methods of Analysis, New York, Chemical Publishing Company, Inc., 1964, p. 439.
3. M. O'Brien, Stepan Laboratory Notebook #0026, pp. 6.

APPENDICES:
Graphics:

Stepan Company Analytical Method

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SM 128-0
Total Pages: 3

Unsaponifiable Matter

Specification Method	Accepted: 02/20/2002	Supercedes:04/22/1997
Author:A. Murphy	Aprv'd: (Anl) (QA)	

SCOPE: This method determines the unsaponifiable matter such as higher aliphatic alcohols, sterols, pigments and hydrocarbons in fatty acids, oils and methyl esters.

SUMMARY: The sample is saponified with potassium hydroxide. The unsaponifiable species are removed by petroleum ether extraction (some fatty acids may be extracted as well). The ether layer is evaporated to dryness and weighed. The fatty acid portion is determined by titrating with sodium hydroxide. The unsaponifiable matter is calculated by subtracting the amount of fatty acids from the residue weight.

SAFETY: 3A ALCOHOL and PETROLEUM ETHER are FLAMMABLE. Avoid open flames and sparks. Work in a hood or well ventilated area.

50% POTASSIUM HYDROXIDE is CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Analytical balance, +/- 0.1mg
2. Stokes flask, 225 mL
3. Erlenmeyer flask, 200 mL
4. Separatory funnel, 500 mL
5. Glass siphon
6. Evaporation flask, 250 mL
7. Cooling condenser,
8. Hot plate/reflux set-up
9. Steam bath
10. Boiling chips
11. Oven, 105 °C, +/- 2 °C

REAGENTS:

1. 3A alcohol, neutral
2. Petroleum ether, reagent grade
3. Potassium hydroxide (KOH), 50% aqueous, wt:wt
4. Sodium hydroxide (NaOH), 0.02N, standardized
5. Phenolphthalein indicator solution
6. 3A alcohol solution, 10% aqueous, vol:vol
7. Deionized water

PROCEDURE: 1. Accurately weigh 5.0g, +/- 0.1g, of sample into an Erlenmeyer flask. Add 30 mL 3A alcohol and 5 mL of 50% KOH, and several boiling chips. Connect the

flask to the condenser and reflux for one hour or until completely saponified.

2. When cooled, transfer the Erlenmeyer flask contents to a Stokes flask (see Remark 1). Rinse the condenser and flask with approximately 20 mL of 3A alcohol. Add rinsings to the Stokes flask. Add approximately 50 mL of cold water to the Stokes flask. The hydrolyzed sample should be in approximately 100 mL of 50:50 3A:water.

3. Cool the Stokes flask contents to room temperature and extract 5 times with petroleum ether, using 30 mL portions each time.

4. The petroleum ether fractions, upper layer, are drawn off with a siphon tube and collected into a 500 mL separatory funnel.

5. Wash the combined extracts with 10% aqueous 3A solution (about 3 times) until the bottom layer is neutral to phenolphthalein.

6. Transfer the washed pet ether layer to a tared evaporation flask and evaporate to dryness on a steam bath under a gentle stream of dry air. Complete the drying process in 105 °C over for 30 minutes. Cool in a desiccator for 20 minutes and weigh. Record this weight as (A).

7. After weighing, dissolve the unsaponifiable matter in 50 mL of warm neutral 3A alcohol. Add a few drops of phenolphthalein and titrate with 0.02N sodium hydroxide to a light pink endpoint.

8. Calculate the free fatty acid content in the residue as stated in Equation 1, CALCULATIONS section.

9. Calculate the unsaponifiable matter as stated in Equation 2, CALCULATIONS section.

CALCULATIONS:

1. Fatty Acid in Residue (B) = NaOH, mL x N NaOH x 0.282 as Oleic Acid

   Where: 0.282 = Oleic Acid Milliequivalent Weight

2. $$\text{Unsaponifiable Matter, \%} = \frac{(A - B) \times 100}{\text{Sample Weight, g}}$$

   Where: A = Residue Weight, g
   B = Fatty Acids Weight,g (see Equation 1)

PRECISION and ACCURACY:

PRECISION:

These error statements are based on an AOCS Smalley Check Sample multilaboratory study updated in 1985-86 on tallow and grease (see Reference 1). The study involved 241 determinations on five samples having an average mean unsaponifiable matter of 0.38 wt.%.

The average standard deviation of the method for between laboratory applications is 26.23 wt.% relative. Two single test results, obtained by different operators in different laboratories on identical test material must agree within 0.28 wt.% absolute.

REMARKS:

1. Alternatively, the Erlenmeyer flask contents can be transferred to a 500 mL

separatory funnel. Collect all rinsings and add to the separatory funnel. This technique will eliminate the siphoning procedure. Draw off the lower aqueous layer into another separatory funnel, retaining the petroleum ether extract in the first funnel. Repeat the petroleum ether extraction of the aqueous layer combining all of the petroleum ether extracts (see Reference 2).

REFERENCES:

1. A.O.C.S. List R-49, AOCS Official Methods Book, Volume 1, 1990.
2. A.O.C.S. Official Method Ca-6a-40.

Graphics:

Stepan Company Analytical Method

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SM 144-0
Total Pages: 3

Sulfated Ash

Specification Method	Accepted: 06/11/2003	Supercedes:08/18/1998
Author:N. Cawley	Aprv'd: (Anl) (QA)	

SCOPE: This method determines the level of inorganic material in surfactants as sulfate.

SUMMARY: The sample is heated in a crucible then ashed in a muffle furnace in the presence of concentrated sulfuric acid. The residual ash is weighed and the percent sulfated ash is calculated.

SAFETY: 3A ALCOHOL is FLAMMABLE. Avoid open flames and sparks. Work in a hood or well ventilated area. Wear proper personal protective equipment.

SULFURIC ACID is CORROSIVE. Avoid eye and skin contact. Work in a hood or well ventilated area. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Analytical balance, +/- 0.0001g or 0.00001g
2. Crucible, porcelain or platinum, with cover
3. Bunsen burner
4. Muffle furnace, capable of 900 °C
5. Gloves, muffle furnace
6. Tongs, stainless steel
7. Desiccator
8. Clay triangle and ring stand

REAGENTS:

1. Sulfuric acid (H2SO4), concentrated
2. Deionized water
3. 3A Alcohol
4. Sea sand, 20-40 mesh

PROCEDURE:

A. MANUAL ASH PROCEDURE:

1. Accurately weigh 1.0-1.5g, +/- 0.0001g, of sample into a clean tared crucible (see Remarks 1, 2, and 3).

2. In a well vented hood, carefully begin ashing the sample with the Bunsen burner.

3. Once the sample begins to smoke, refrain from concentrating the flame directly on the crucible for any period of time to avoid splattering of the sample (see Remark 4). As the smoke subsides, reapply heat to the crucible.

4. Continue carefully ashing until the sample has formed a "crusty" residue, then

increase the intensity of the heating so the crucible glows a dull red color.

5. Allow the crucible to cool, then add 3-5 drops of concentrated sulfuric acid.

6. Reapply heat, taking care not to heat the crucible so quickly as to cause splattering of the sulfuric acid and sample (see Remark 5). Continue to heat until the sulfuric acid stops fuming.

7. Repeat the sulfuric acid addition 2-4 times until the residue is white to grayish white in color.

8. Using the muffle furnace gloves and tongs, carefully place the crucible in the muffle furnace at 900 °C for 1 hour. Do not contaminate the crucible with material from the walls of the muffle furnace.

9. Carefully remove the crucible from the muffle furnace and place in a desiccator to cool for 45-60 minutes.

10. Accurately reweigh the crucible and residue contents. Calculate the percent sulfated ash as stated in the CALCULATIONS section.

B. AUTOMATED ASH PROCEDURE:

1. Accurately weigh 1.0-1.5g, +/- 0.0001g, of sample into a clean tared crucible (see Remarks 1, 2, and 3).

2. Add 3-5 drops of concentrated sulfuric acid to the sample.

3. Place the crucible in the muffle furnace and program using the following conditions:

Initial Temp	25-100 °C
Rate 1	4 °C/minute
Temp 1	500 °C
Hold Time	0 minutes
Rate 2	10 °C/minute
Temp 2	900 °C
Hold Time	20 minutes

4. When the ashing is complete, carefully remove the crucible and place in a desiccator to cool for approximately 45-60 minutes.

5. Accurately reweigh the crucible and residue contents. Calculate the percent sulfated ash as stated in the CALCULATIONS section.

CALCULATIONS:

$$\text{Sulfated Ash, \%} = \frac{(B - A) \times 100}{\text{Sample Weight, g}}$$

Where: A = Crucible Tare Weight, g
B = Crucible and Ash Weight, g

PRECISION and ACCURACY:

PRECISION:

Ten replicate determinations were performed on 2 samples by 2 analysts in 1 laboratory. Results are considered acceptable if the standard deviation is not

greater than 0.1% and the individual difference from the mean is not greater than 0.01%.

REMARKS:

1. Clean the crucible by mixing 1-2g of sea sand with a few mL of deionized water and mix thoroughly. Rinse the crucible with water or a suitable alcohol and let dry.
2. To accurately weigh a sample, it is necessary that the analytical balance be accurate to a minimum of 0.0001g and be located on a solid surface free from vibration. The area must also be free from drafts. It is necessary to zero the balance before each weighing.
3. Prior to the analysis, bring the crucible to constant weight by heating in the muffle furnace at 900 °C for 1 hour. This will insure that the crucible is clean.
4. Ignition of the sample is permissible since the free oil may have a tendency to catch fire. However, an excessively large flame should be extinguished to avoid the release of any soot.
5. Avoid skin contact and inhalation of the corrosive sulfuric acid fumes.

Graphics: